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Rodent Models of Varicella-Zoster Virus Neurotropism

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Abstract

Inoculation of rodents with varicella-zoster virus (VZV) results in a latent infection in dorsal root ganglia with expression of at least 5 of the 6 VZV transcripts and one of the viral proteins that are reported to be expressed during latency in human ganglia. Rats develop allodynia and hyperalgesia in the limb distal to the site of injection and the resulting exaggerated withdrawal response to stimuli is reduced by treatment with gabapentin and amitryptyline, but not by antiviral therapy. Inoculation of rats with VZV mutants show that most viral genes are dispensable for latency, but that some genes (e.g. ORF4, 29 and ORF63) which are expressed during latency are important for establishment of latency in rodents, but not for infection of rodent ganglia. The rodent model for VZV latency allows one to study ganglia removed immediately after death, avoiding the possibility of reactivation, and helps to identify VZV genes required for latency.

1 Varicella-zoster virus (VZV) latency in humans

Varicella-zoster virus (VZV) establishes latency in human cranial nerve and dorsal root ganglia (Mahalingam et al. 1990). Viral DNA is present in the neurons and a limited set of VZV genes are expressed: ORF4, ORF21, ORF29, ORF62, ORF63, and ORF66 (Cohrs et al. 2003; Grinfeld and Kennedy 2004; Kennedy et al. 2000; Lungu et al. 1998; Mahalingham et al. 1996). Both viral transcripts and proteins have been detected in the cells. Interestingly during latency, ORF4, 21, 29, 62, and 63 proteins have been detected in the cytoplasm of neurons (Grinfeld and Kennedy 2004; Lungu et al. 1998; Mahalingham et al. 1996), while during reactivation the proteins were detected in both the cytoplasm and the nucleus (Lungu et al. 1998). In contrast, several viral proteins, glycoprotein C (gC), gI and ORF10 were not detected in latently infected human neurons (Lungu et al. 1998).

2 VZV latency in rats

Initial studies in adult rats showed that injection of VZV-infected cells subcutaneously along the side of the spine resulted in latent infection in the dorsal root ganglia innervating the site of injection (Sadzot-Delvaux et al. 1990). VZV nucleic acid was detected by in situ hybridization one to 9 months after inoculation in neurons in the dorsal root ganglia. VZV proteins were detected in neurons using human sera, and viral proteins were only detected in the cytoplasm of the neurons. In one set of experiments, serial repeated passages of latently infected ganglia in human fibroblasts eventually resulted in cytopathic effects consistent with VZV in fibroblasts. Similar reactivation of VZV with infectious virus ex vivo has not been was reported subsequently. In a follow-up study by the same authors, VZV DNA was detected only in the dorsal root ganglia corresponding to the side of the spine injected; however, sectioning of the nerve roots before inoculation still allowed infection of the ganglia, but at a lower level of infection (Sadzot-Delvaux et al. 1995). In situ hybridization showed VZV in both neurons and nonneuronal cells.

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Subsequent experiments from other researchers showed that VZV nucleic acid could be detected by in situ hybridization in dorsal root ganglia of rats inoculated in the foot pad (Annunziato et al. 1998; Kennedy et al. 2001) or subcutaneously along the skin (Annunziato et al. 1998). In one report, VZV nucleic acid was detected in both neurons and satellite cells, and only in the nucleus of neurons (Annunziato et al. 1998). VZV in situ hybridization was positive in bilateral dorsal root ganglia, even though only one side of the spine or foot pad was infected, suggesting that viremia had resulted with seeding of the dorsal root ganglia. No difference was noted in the frequency of latent infection in animals vaccinated with the Oka vaccine strain and VZV strain Ellen or in animals inoculated one month or 3 months before ganglia were obtained. In another report, VZV RNA was detected in neurons and noneuronal cells at a ratio of 3:1, and only in the nucleus of neurons (Kennedy et al. 2001). VZV RNA was detected in dorsal root ganglia at one week, one month, and 18 months after infection; similar numbers ganglia were positive for VZV at one and 18 months. VZV was not detected in the liver, lung, or kidney after establishment of latency in dorsal root ganglia, but was detected in the spleen in one of three rats (Grinfeld et al. 2004).

2.1 VZV latency in newborn rats and other rodents

Intraperitoneal inoculation of newborn rats with VZV resulted in a latent infection of the trigeminal ganglia with viral DNA and RNA detected five to six weeks after infection (Brunell et al. 1999). Inoculation of cotton rats with VZV-infected cells along the side of the spine resulted in latent infection of dorsal root ganglia (Sato et al. 2002b). Latency was not established in animals infected with heat-inactivated VZV-infected cells.

Corneal inoculation of mice resulted in latent infection in the trigeminal ganglia as well as viral DNA in the brainstem, kidneys, spleen, and liver by nested PCR at one month after infection (Wroblewska et al. 1993). In situ hybridization showed VZV RNA in trigeminal ganglia and spleen of mice one month after infection. Viral RNA was present at a higher frequency in nonneuronal cells than in neurons.

2.2 VZV allodynia model in rats

Inoculation of VZV-infected cells into the footpad of rats resulted in allodynia and hyperalgesia in the ipsilateral limb (Fleetwood-Walker et al. 1999). Animals were tested for paw withdrawal after exposure to nylon microfilaments or to heat. The effects persisted for up to 33 days after injection. In contrast, no neurologic changes were noted in the contralateral limb. VZV IE63 protein, but not inflammatory cells, was detected in the ipsilateral dorsal root ganglia. The authors concluded that the neurological changes in the rats might serve as a model for post-herpetic neuralgia.

A follow-up study by the same research group showed that allodynia began within 3 days of inoculation of VZV-infected cells, persisted at least 30 days, and resolved within 100 days (Dalziel et al. 2004). Treatment of animals with valacyclovir for the first 10 days after infection had no effect on allodynia implying that continued virus replication is not required for the neurologic effects. Inoculation of the footpad of rats with herpes simplex virus (HSV) resulted in hyperalgesia on days 1 to 4 after injection and the neurologic effects resolved by day 6. Some animals developed hindlimb paralysis after HSV infection.

Examination of dorsal root ganglia from rats infected with VZV showed increased expression of neuropeptide Y, galanin, and ATF-3, a marker of nerve injury (Garry et al. 2005). Treatment of rats with gabapentin, sodium channel blockers (mexiletine and lamotrigine), but not a nonsteroidal anti-inflammatory drug (diclofenac) reduced the paw withdrawal response to mechanical and noxious thermal stimuli. Testing of different strains of VZV showed a dose response to mechanical, but not thermal stimuli (Hasnie et al. 2007).

Treatment of rats with morphine, amitryptyline, ibuprofen, a cannabinoid (Hasnie et al. 2007), a palmitoylethanolamide analogue (Wallace et al. 2007), and histamine H3 receptor antagonists (Medhurst et al. 2008) resulted in a reduced paw withdrawal in response to mechanical stimuli.

3 VZV transcripts in latently infected rats and cotton rats

VZV transcripts corresponding to ORF4, 29, 62, and 63 were detected by Sadzot-Delvaux (1995) and colleagues in adult rats latently infected with VZV (Table 1). No transcripts were detected for the thymidine kinase or glycoprotein E genes in latently infected rats. Northern blotting showed that transcripts for ORF62 and ORF63 were of the same length in latently infected animals as in infected cells in culture. VZV ORF21 and ORF63 were detected by in situ hybridization in dorsal root ganglia of rats one month after infection, and ORF62 and ORF63 were detected 18 months after infection (Kennedy et al. 2001). One dorsal root ganglia was positive for ORF29, and one each for ORF18 and for ORF40 (the latter two genes have not associated with latency in humans) at one to 18 months after infection. In another study by the same authors, 39% of latently infected rat dorsal root ganglia were positive for ORF62 RNA, 35% for ORF63, 25% for ORF4, 25% for ORF 29, 24% for ORF18, 22% for ORF28, 13% for ORF21, and 0% for ORF40 (Grinfeld et al. 2004). ORF21, but not ORF40 RNA was detected by reverse transcriptase PCR in latently infected newborn rats (Brunell et al. 1999). VZV ORF63 RNA was detected in dorsal root ganglia in 25 of 36 latently infected cotton rats by reverse transcriptase-PCR one month after infection; in contrast, ORF40 RNA was detected in only one of 36 animals (Sato et al. 2002b).

One concern raised about detection of multiple VZV transcripts in humans is whether these represent transcripts during latency or whether the virus may reactivate between the time of death and when the ganglia are studied. To address this issue, Grinfeld and colleagues (2007) assayed VZV RNA transcripts by in situ hybridization and by nested reverse-transcriptase PCR in latently infected rat ganglia 18 months after infection at various times after death of the animal. VZV ORF63 transcripts were detected in 48%, 67%, and 42% of ganglia at 0, 24, and 48 hours after death, respectively, while ORF40 transcripts were detected in 9–10% of animals 0, 24, and 48 hours after death. Thus, there was no increase in the percentage of ganglia expressing VZV transcripts and these results argue against VZV reactivation between the time of death and when the ganglia are examined.

4 VZV proteins in latently infected rats

VZV proteins have been detected in dorsal root ganglia from rats infected with VZV. IE63 was detected in 50% to 80% of neurons in dorsal root ganglia from latently infected rats using rabbit antibody to IE63 (Debrus et al. 1995; Sadzot-Delvaux et al. 1995) (Table 1). IE63 was detected in both the nucleus and cytoplasm of the neurons, and some noneuronal cells and axons also contained IE63. VZV IE62, ORF29 protein, and glycoprotein E could not be detected in latently infected dorsal root ganglia. IE63 was also detected in latently infected dorsal root ganglia of rats using a monoclonal antibody to IE63 at one and 18 months after infection (Kennedy et al. 2001). IE63 was detected in both the nucleus and cytoplasm of the neurons with the monoclonal antibody.

5 VZV genes important for latency in rats and cotton rats

The observation that infection of rats or cotton rats with VZV, either in the foot pad or along the side of the spine, results in latent infection of dorsal root ganglia has allowed many VZV mutants to be studied for their ability to establish latency (Table 2).

5.1 Role of VZV genes not conserved with HSV in establishment of latency in rodents

VZV apparently lacks a latency associated transcript (LAT) similar to that in HSV and many other alphaherpesviruses including pseudorabies virus, bovine herpesvirus and equine herpesvirus. Deletion of the LAT has been associated with reduced establishment of latency in mice in some (Thompson and Sawtell, 2001), but not all (Leib et al. 1989) experiments. Since VZV encodes six genes (ORF 1, 2, 13, 32, 57, S/L) that lack homologs in HSV, we tested whether some of these genes might be important for establishment of latency. VZV unable to express ORF1, 2, 13, 23, or 57 is not impaired for growth in cell culture (Cohen and Seidel 1993; Cohen and Seidel 1995; Cox et al. 1998; Reddy et al. 1998; Sato et al. 2002b); each of the VZV mutants established latency at similar levels as parental virus (Sato et al. 2002b; Sato et al. 2003b). Both the number of animals with latent infection and the geometric mean of VZV genome copies for VZV-positive ganglia were similar in animals infected with the mutant and wild-type viruses.

5.2 Role of VZV genes expressed during latency in humans in establishment of latency in rodents

VZV encodes six genes (ORF4, 21, 29, 62, 63, and 66) that are expressed during latency in humans (Cohrs et al. 2003; Grinfeld and Kennedy 2004; Kennedy et al. 2000; Lungu et al. 1998; Mahalingham et al. 1996). ORF4 encodes a transcriptional activator that is essential for replication of VZV in vitro; VZV deleted for ORF4 could only be grown in cells expressing ORF4 protein (Cohen et al. 2005b). VZV deleted for ORF4 was passaged once in non-complementing cells so that the no ORF4 protein would be present at the time of infection. Inoculation of cotton rats with cells containing the ORF4 deleted virus resulted in latent infection of the dorsal root ganglia. The frequency of animals with latent infection was lower in those infected with the ORF4 deletion mutant than with wild-type virus. Examination of dorsal root ganglia of cotton rats three days after inoculation with the ORF4 deletion mutant showed that most of the animals had viral DNA in the ganglia. Therefore VZV deleted for ORF4 was impaired for establishing latency, but not for entering ganglia.

VZV ORF21 encodes a nucleotide protein and ORF21 transcripts and protein are expressed in latently infected human and rodent ganglia (Grinfeld and Kennedy 2004; Kennedy et al. 2000; Lungu et al. 1998). VZV deleted for ORF21 cannot replicate in cell culture unless grown in cells expressing ORF21 (Xia et al. 2003). Inoculation of cotton rats with cells expressing ORF21 that were infected with the ORF21 deletion mutant resulted in latent infection in a similar number of animals, with a similar copy number of VZV genomes in VZV-positive ganglia, as infection of animals with wild-type virus. The ORF21 deletion mutant had been grown in ORF21 expressing cells immediately before infection of the animals and ORF21 protein may have been present when the ganglia were infected.

VZV ORF29 encodes a single-stranded DNA binding protein that is expressed during latency in human and rodent ganglia (Grinfeld and Kennedy 2004; Kennedy et al. 2000; Lungu et al. 1998). ORF29 is essential for replication of the virus in cell culture and could only be grown in cells expressing ORF29 (Cohen et al 2007). Inoculation of cotton rats with cells infected with ORF29 deleted virus, which had been passaged once in the absence of cells expressing the protein, showed that VZV deleted for ORF29 is severely impaired for establishment of latency in cotton rats (Cohen et al 2007). Examination of dorsal root ganglia from animals inoculated with VZV deleted for ORF29 three days after infection showed that the deletion mutant was present in similar numbers of ganglia as wild-type virus; thus ORF29 is not required for entry into ganglia. Interestingly, expression of ORF29 by the human cytomegalovirus promoter, instead of its own promoter, also resulted in impaired latency in cotton rats.

ORF66 transcripts and protein have been detected in latently infected human ganglia (Cohrs et al 2003). ORF66 encodes a viral protein kinase that phosphorylates VZV IE62 resulting in its incorporation into the virion. ORF66 is dispensable for infection in vitro (Heineman et al. 1996). ORF66 is important for growth of VZV in human T cells (Moffat et al. 1999, Soong et al. 2000), but is dispensable for establishment of latency in cotton rats (Sato et al. 2003b).

ORF63 is located in both internal repeats of the VZV genome, inhibits expression of ORF62 (Hoover et al. 2006), and inhibits the activity of interferon alpha (Ambagala et al 2007). ORF63 transcripts and protein are expressed in latently infected human ganglia (Grinfeld and Kennedy 2004; Kennedy et al. 2000; Lungu et al. 1998, Mahlingham et al.1996). VZV deleted for both copies of ORF63 was severely impaired for replication in vitro. Inoculation of the ORF63 deletion mutant into cotton rats resulted in fewer animals with latent infection and fewer VZV genome copies in latently infected ganglia compared to wild-type virus (Cohen et al 2004). VZV deleted for ORF63 could enter ganglia since high levels of VZV DNA were present in ganglia three days after infection; however, by days 6 and 10 after infection the numbers of viral genomes had rapidly declined.

A series of carboxyl terminal truncation mutations of ORF63 showed that deletion of the last 70 amino acids did not impair growth in cell culture or establishment of latency in cotton rats; in contrast, deletion of the last 108 amino acid impaired growth in vitro and latency in rodents (Cohen et al. 2005a). Substitution of five serine or threonine putative phosphorylation sites in ORF63 with alanine also impaired the growth of the virus in vitro and latency in cotton rats.

5.3 Role of other VZV genes in establishment of latency in rodents

VZV ORF61 is a transcriptional activator that upregulates expression from immediate-early and putative early promoters. A deletion mutant of ORF61 is impaired for replication in vitro (Cohen and Nguyen, 1998), but ORF61 is dispensable for establishment of latency (Sato et al. 2003a). VZV ORF47 encodes a viral protein kinase that phosphorylates several viral proteins and is required for replication in human T cells and skin (Moffat et al. 1999; Soong et al. 2000), but is not required for replication in vitro (Heineman and Cohen, 1995) or for latency in cotton rats (Sato et al. 2003b). VZV ORF10 is a transcriptional activator that is not required for VZV replication in vitro (Cohen and Seidel 1994). While HSV-1 VP16, the homolog of ORF10, is unable to establish latency in mice (Tal-Singer et al., 1999), VZV ORF10 is dispensable for latency in cotton rats (Sato et al. 2003b). VZV ORF17 induces RNA degradation in cells (Sato et al 2002a). While VZV deleted for ORF17 is impaired for growth at 37°C, the mutant virus was not impaired for latency in cotton rats which have a core temperature of 39°C. Interestingly, HSV-1 deleted for vhs, the homolog of ORF17, is impaired for establishment of latency (Strelow and Leib, 1995).

VZV ORF14 encodes glycoprotein C which is dispensable for replication in cultured cells, but is critical for replication in human skin (Moffat et al. 1998). VZV deleted for glycoprotein C was not impaired for establishment of latency in rats (Grinfeld et al. 2004). VZV ORF67 encodes glycoprotein I which is required for virus replication in human skin and T cells (Moffat et al. 2002); however, glycoprotein I is dispensable for latency in rats (Grinfeld et al. 2004).

5.4 Summary of latency studies in rodents

Analysis of the growth of VZV mutants in vitro and their ability to establish latent infection in rodents indicates that some mutants that are severely impaired for growth in cell culture are not impaired for establishment of latency (ORF17 and ORF61 deletion mutants), while other mutants impaired for growth in culture are impaired for latency (ORF63 deletion

mutant). All of the mutants are able to infect ganglia and establish latency, including some that are unable to replicate in cell culture in the absence of complementing cells (ORF4 and ORF29 deletion mutants). Some (ORF4 and 29 deletion mutants), but not all (ORF66 stop codon mutant) mutants in genes expressed during latency are impaired for establishment of latency.

6 Advantages and limitations of the rodent model of VZV latency

These are several advantages of the rodent model for studying VZV latency (Table 3). One of the concerns of latency studies with human ganglia is that nervous system tissues are often removed hours to days after death and reactivation of VZV may have occurred which might be confused with latent infection. The rodent model allows ganglia to be removed immediately after death and avoid concerns about reactivation. Studies in animals allow investigation of various mutant viruses that can determine the role of individual viral genes in latency. In addition, viruses labeled with beta-galactosidase (Cohen et al. 1998), green fluorescence protein (Zerboni et al. 2000, Li et al. 2006), and luciferase (Oliver et al. 2008, Zhang et al. 2007) might be used to identify latently infected neurons.

The rodent model of VZV latency has several limitations compared with the human system (Table 3). First, VZV is not a natural pathogen in rodents and therefore the virus has not evolved with the immune system of the animal. Viral proteins that change in response to human host defenses or that are pirated from host genes may differ in their activity in rodents when compared to humans. Second, animals do not develop chickenpox after infection and therefore viremia, with spread of virus to distant ganglia is probably more limited in rodents than in humans. However, dissemination of virus to ganglia not directly innervating the site of inoculation has been demonstrated (Annunziato et al. 1998; Brunell et al. 1999). Third, reactivation of VZV has not been detected in vivo in rodents or in explanted ganglia from these animals, with one possible exception (Sadzot-Delvaux et al. 1990). It is important to note that while VZV causes zoster in up to 50% of persons who live to age 85, reactivation has never been demonstrated in explanted human ganglia. Since reactivation in humans most frequently occurs after the 6^{th} decade of life, and rodents are usually tested a few months to a year after infection, it is possible that insufficient time has occurred to demonstrate reactivation. However, studies with immunosuppressed animals have also failed to show reactivation (Cohen et al, unpublished data).

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Abbreviations

VZV varicella-zoster virus

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Table 1

VZV genes and proteins expressed during latency in rodents

Transcript/protein	Animal	Reference
ORF4 RNA	rat	Sadzot-Delvaux et al. 1995
	rat	Grinfeld et al. 2004
ORF21 RNA	neonatal rat	Brunell et al. 1996
	rat	Kennedy et al. 2001
	rat	Grinfeld et al. 2004
ORF29 RNA	rat	Sadzot-Delvaux et al. 1995
	rat	Kennedy et al. 2001
	rat	Grinfeld et al. 2004
ORF62 RNA	rat	Sadzot-Delvaux et al. 1995
	rat	Kennedy et al. 2001
	rat	Grinfeld et al. 2004
ORF63 RNA	rat	Sadzot-Delvaux et al. 1995
	rat	Kennedy et al. 2001
	rat	Grinfeld et al. 2004
	cotton rat	Sato et al. 2002b
ORF18 RNA *	rat	Grinfeld et al. 2004
ORF28 RNA *	rat	Grinfeld et al. 2004
ORF40 RNA *	rat	Grinfeld et al. 2007
ORF63 protein	rat	Debrus et al. 1995
	rat	Sadzot-Delvaux et al. 1995
	rat	Kennedy et al. 2001

* These transcriptase have not generally been detected in latently infected human ganglia; ORF40 transcripts were absent or detected in less than 3% of rodents in other studies (Brunell et al 1999; Sato et al 2002 [ORF2]).

Table 2

VZV Genes tested for latency in cotton rats or rats

VZV Gene	Mutation	Phenotype in Latency	Reference
ORF1	stop	dispensable	Sato et al. 2003b
ORF2	del	dispensable	Sato et al. 2002b
ORF4	del	impaired	Cohen et al. 2005b
ORF10	del	dispensable	Sato et al. 2003b
ORF13	stop	dispensable	Sato et al. 2003b
ORF14	del	dispensable	Grinfeld et al. 2004
ORF17	del	dispensable	Sato et al. 2002a
ORF21	del	dispensable *	Cohen et al. 2003
ORF29	del	impaired	Cohen et al. 2007
	ectopic promoter	impaired	Cohen et al. 2007
ORF32	del	dispensable	Sato et al. 2003b
ORF47	stop	dispensable	Sato et al. 2003b
ORF57	del	dispensable	Sato et al. 2003b
ORF61	del	dispensable	Sato et al. 2003a
ORF63	del	impaired	Cohen et al. 2004
	carboxy terminal truncations	dispensable or impaired	Cohen et al. 2005a
	phosphorylation sites	impaired	Cohen et al. 2005a
ORF66	stop	dispensable	Sato et al. 2003b
ORF67	stop	dispensable	Grinfeld et al. 2004

* virus was not passaged in non-complementing cells before testing for latency Stop=stop codon, del=deletion, ectopic promoter=ORF29 expressed under a cytomegalovirus promoter

Table 3

Advantages and limitations of rodent models for VZV latency compared with studies in humans

Advantages	
1	Ganglia can removed immediately after death to avoid the possibility of reactivation post mortem
2	Virus mutants can be studied to determine the role of individual genes in latency.
3	Viruses labeled with markers might be used to identify latently infected cells.
Limitations	
1	VZV is not a natural pathogen of rodents and viral proteins may not function the same in rodents and in humans.
2	Viremia appears to be limited in rodents infected with VZV
3	VZV reactivation has not occurred in rodents in vivo.